Journal of Experimental Botany, Vol. 57, No. 14, pp. 3793–3799, 2006
doi:10.1093/jxb/erl140 Advance Access publication 22 September, 2006
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Journal of Experimental Botany

RESEARCH PAPER

Effect of activase level and isoform on the thermotolerance of photosynthesis in *Arabidopsis*

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Received 30 May 2006; Accepted 26 July 2006

Abstract

Ribulose 1,5-*bis*phosphate carboxylase/oxygenase (Rubisco) activation decreases under moderate heat stress. This decrease is caused by an impairment of activase function, which is exacerbated by faster rates of Rubisco deactivation at elevated temperatures. To determine if stromal oxidation causes inhibition of activase, transgenic *Arabidopsis* plants expressing suboptimal amounts of either the redox-regulated 46 kDa α- or non-redox regulated 43 kDa β-isoform of activase were examined. Photosynthesis, as measured by gas exchange and chlorophyll fluorescence, and Rubisco activation were inhibited to a much greater extent by moderately high temperatures in the two transgenic lines expressing suboptimal levels of the individual isoforms of activase compared with wild-type plants or transgenic plants expressing levels of the β-isoform sufficient for wild-type rates of photosynthesis. Net photosynthesis and Rubisco activation in transgenic plants expressing suboptimal amounts of the β-isoform of activase from the Antarctic hairgrass were even more sensitive to inhibition by moderate heat stress than in the transgenic plants containing *Arabidopsis* activase. The results demonstrate that photosynthesis exhibits a similar sensitivity to inhibition by moderately high temperature in plants expressing either of the two different isoforms of activase. Thus, impairment of activase function under heat stress is not caused by oxidation of the redox-sensitive sulphydryls of the α-isoform of activase. Instead, the results are consistent with thermal denaturation of activase under moderate heat stress, the effects of which on Rubisco activation would be enhanced when activase levels are suboptimal for photosynthesis.

Key words: Carbon metabolism, heat stress, photosynthesis, Rubisco, temperature stress.

Introduction

Photosynthesis is extremely susceptible to inhibition by moderate heat stress (Berry and Björkman, 1980). Of the components of photosynthesis that are impaired as temperature increases, ribulose 1,5-*bis*phosphate carboxylase/oxygenase (Rubisco) activation has long been recognized as one of the most sensitive (Weis, 1981*a*, *b*; Kobza and Edwards, 1987). At permissive temperatures, Rubisco is maintained in an active state by the continued action of activase, a molecular chaperone that converts Rubisco from an inactive closed conformation to an active open conformation (Andrews, 1996; Spreitzer and Salvucci, 2002; Portis, 2003). Processes that inactivate Rubisco, including the synthesis of catalytic misfire products (Edmondson *et al*., 1990; Kane *et al*., 1998), increase with temperature to at least 50 °C (Salvucci and Crafts-Brandner, 2004*b*), whereas the ability of activase to reverse inactivation exhibits a relatively low temperature optimum (Eckhardt and Portis, 1997; Crafts-Brandner and Salvucci, 2002; Salvucci and Crafts-Brandner, 2004*a*, *c*). Consequently, Rubisco deactivates at elevated temperatures because increased rates of Rubisco deactivation are not offset by faster rates of reactivation by activase (Crafts-Brandner and Salvucci, 2002).

Activase catalyses ATP hydrolysis (Robinson and Portis, 1989), an activity that is required for it to act as chaperone towards Rubisco (Shen *et al*., 1991; Salvucci and Klein, 1994). Since ATP hydrolysis is inhibited by ADP, Rubisco activation by activase is responsive to changes in the ATP/ADP ratio (Robinson and Portis, 1989; Zhang and

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Abbreviations: RuBP, ribulose 1,5-bisphosphate; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase.

Portis, 1999). In many plant species, the relative sensitivity of activase to inhibition by ADP depends on the redox status of the longer, α-form of activase. This form of activase, which is not present in some plant species (Salvucci *et al*., 1987), contains two cysteine residues near the C-terminus that can be oxidized to form a disulphide (Zhang and Portis, 1999). Reduction of this disulphide by thioredoxin-f decreases the sensitivity of the activase holoenzyme to inhibition by ADP, making activase responsive to changes in irradiance via its sensitivity to the redox status of the chloroplast (Zhang *et al*., 2002).

Estimates of the redox state of chloroplasts in heat-stressed leaves, determined from changes in the activation state of malate dehydrogenase, indicated that the chloroplast stroma becomes more oxidized under moderate heat stress (Schrader *et al*., 2004). This finding led to the suggestion of a cause and effect relationship between stromal oxidation and Rubisco inactivation. Studies by Kim and Portis (2005) with transgenic *Arabidopsis* plants appeared to lend support to this idea by showing that photosynthesis in plants expressing only the redox-regulated α-isoform of activase was more sensitive to inhibition by moderate heat stress than photosynthesis in the wild type or in plants expressing only the non-redox regulated β-isoform. However, the steady-state levels of activase were limiting for photosynthesis in the plants that expressed only the α-isoform, but not in the plants that only expressed the β-isoform. As the authors indicated, differences in the amount rather than the form of activase might explain the difference in thermotolerance between transgenic lines that expressed the different forms of activase.

To determine the role of stromal oxidation and activase amount in the inactivation of Rubisco, a transgenic *Arabidopsis* line, Δ43, was produced that expresses lower amounts of the non-redox-regulated β-isoform of activase. For additional comparison, transgenic plants were also produced that expressed the β-isoform of activase from the Antarctic hairgrass, *Deschampsia antarctica*. Activase from *D. antarctica* was previously shown to have an exceptionally low temperature optimum for activity (Salvucci and Crafts-Brandner, 2004*c*). The temperature response of photosynthesis and Rubisco activation was determined in plants from these two lines and compared with the responses of wild-type plants and the transgenic plants described previously by Kim and Portis (2005). The results are inconsistent with an involvement of stromal oxidation in the loss of Rubisco activation under moderate heat stress.

Materials and methods

Plant material

Transgenic *Arabidopsis thaliana* plants expressing near wild-type levels of the β-isoform and suboptimal levels of the α-isoform have been described previously (Zhang *et al*., 2002; Kim and Portis, 2005). *Arabidopsis* plants expressing suboptimal levels of the *Arabidopsis* and *D. antarctica* β-isoform of activase were produced by *Agrobacterium*-mediated transformation of the activase-deficient *rca* mutant. A full-length cDNA for the coding region of the β-isoform of activase from these species (Salvucci *et al*., 2004*c*) containing the *Arabidopsis* activase transit peptide was constructed using polymerase chain reaction (PCR). The *Arabidopsis* cDNA was engineered to include nucleotides that encoded an eight amino acid S-tag (Skerra and Schmidt, 2000) attached to the C-terminus via a serine–alanine linker. The resulting cDNAs were cloned into the binary expression vector pMDC32 (Curtis and Grossniklaus, 2003) via the pENTR/D-TOPO vector using Gateway clonase technology, which is based on bacteriophage λ site-specific recombination (Invitrogen, Carlsbad, CA, USA). After confirmation by nucleotide sequencing, expression cassettes were transferred into *Agrobacterium* strain GV3850. *Arabidopsis* plants were transformed using a floral dip method (Clough and Bent, 1998). Positive transformants were identified by selecting kanamycin-resistant seedlings. T3 lines homozygous for a single active T-DNA insert were used for further experiments. Wild-type and transgenic plants were grown in air at 23 °C under an irradiance of 250 μmol photons m^{-2} s^{-1} with a 10 h light/14 h dark photoperiod.

Gas exchange and fluorescence measurements

The gas exchange of attached leaves was measured at air levels of CO_2 (internal CO_2 ~280 μbar) and either 202 or 9.6 mbar O_2 on 4–8-week-old plants (see text) using a Li-Cor 6400 as described previously (Salvucci and Crafts-Brandner, 2004*c*). Control plants were measured after at least 1 h at 23 °C. Heat stress was imposed by increasing the temperature of the growth chamber and the leaf cuvette from 23 °C to 37.5 °C over a 15 min period and then measuring gas exchange after 1 h at the higher temperature. This temperature was sufficient to cause moderate (<50%) inhibition of net photosynthesis in wild-type plants (see below). The relative humidity in the chamber was maintained at 80% during the treatment to ensure that leaf temperatures were similar to air temperatures. Chlorophyll fluorescence of attached leaves was measured at 250 μmol photons m^{-2} s^{-1} as described previously (Salvucci and Crafts-Brandner, 2004*c*). For fluorescence measurements, the temperature of the growth chamber was increased in increments of 2–5 °C. Fluorescence measurements were made after 20 min at each temperature. The results presented are the means ±SEM of individual measurements on 6–10 separate plants.

Rubisco activation

Initial and total Rubisco assays were conducted at 25 °C as described previously (Salvucci *et al*., 1986; Salvucci and Crafts-Brandner, 2004*c*), except that 1 mM phenylmethylsulphonyl fluoride (PMSF) and 10 μM leupeptin were included in the extraction buffer. Leaf discs were excised from intact plants and immediately frozen in liquid N_2. Heat stress was imposed as described above for the gas exchange measurements and plants were sampled after 1 h of exposure to control or high temperature. Duplicate assays were conducted on each sample, each sample consisting of two discs each from a separate plant. The results presented are the means ±SEM of 3–5 samples.

Protein determination and analysis

Soluble protein was determined by a dye binding assay (Bradford, 1976). Methods for SDS–PAGE and immunoblots have been described previously (Salvucci *et al*., 2001). The levels of activase in leaf extracts of Δ43 plants were determined by image analysis of immunoblots using serial dilutions of purified recombinant *Arabidopsis* and *D. antarctica* activase as standards. Recombinant activase was purified from *Escherichia coli* cells as described previously (Salvucci and Crafts-Brandner, 2004*c*). For immunoblots, leaf discs

were collected on the same day that gas exchange and Rubisco activation measurements were conducted and from the same cohort of plants. Discs were extracted in the same extraction buffer used for the activity measurements.

Results

Activase levels and plant growth

Wild-type *Arabidopsis* plants express approximately equal amounts of the 46 kDa redox-regulated α- and 43 kDa non-redox-regulated β-isoforms of activase (Fig. 1; Salvucci *et al*., 1997). The transgenic lines rwt46 and rwt43 described previously (Kim and Portis, 2005) expressed reduced amounts of the α- and β-isoforms of *Arabidopsis* activase, respectively. The amount of activase in the rwt46 plants, 51% of the total amount in wild type, was insufficient to support wild-type rates of photosynthesis, whereas the amount in rwt43, 71% of the total amount in wild type, was adequate for wild-type rates of net photosynthesis (Zhang *et al*., 2002; also see below).

The transgenic line, Δ43, constructed for this study expressed reduced amounts of the β-isoform of *Arabidopsis* activase relative to the level of β-isoform in the wild type. The amount of activase in the leaves of Δ43 plants was ~12% of the total (i.e. α- plus β-isoform) amount in wild-type leaves based on immunoblots (Fig. 1). The transgenic line, ΔDa, also expressed reduced amounts of just the β-isoform of activase, but the activase was from the Antarctic hairgrass, *D. antarctica* (Salvucci *et al*., 2004*c*). The amount of *D. antarctica* activase in ΔDa plants was ~10% of the total amount of activase in wild-type *Arabidopsis* based on immunoblots.

The Δ43 plants grew well in air, although their growth was slower than the wild type under the short day growth conditions used in this study. Above-ground biomass for 1-month-old plants was 1.56±0.14 and 0.67±0.03 g^{-1} FW plant or 0.112 and 0.031 g^{-1} DW plant for wild-type and Δ43 plants, respectively. At this stage of development, the difference in biomass between wild type and the Δ43 plants represented <1 week of growth. The ΔDa plants that expressed activase from *D. antarctica* looked normal and reached a similar size to the wild type, but grew much more slowly than the wild type. Consequently, these plants required 8 weeks to accumulate the equivalent amount of biomass to 4-week-old wild-type plants.

Response of photosynthesis to moderate heat stress

Net photosynthesis in air at 23 °C was similar in the wild type and the transgenic line, rwt43, but reduced in the transgenic line rwt46 (Table 1). The Δ43 plants exhibited photosynthetic rates that were only 53% of the wild type, while the ΔDa plants had even lower photosynthetic rates. Thus, at the control temperature of 23 °C, net photosynthesis was limited by the amount of activase in Δ43 and rwt46 plants, the two transgenic lines with reduced amounts of *Arabidopsis* activase, and in ΔDa transgenic *Arabidopsis* plants that expressed reduced amounts of the *D. antarctica* activase.

Fig. 1. Immunoblot of leaf extracts from wild-type (wt) and transgenic (rwt43, rwt46, and Δ43) *Arabidopsis* plants. An equal amount of leaf tissue from each plant line was extracted in buffer, and the polypeptides were separated by SDS–PAGE and transferred to a polyvinylidene fluoride membrane. Blots were probed with antibody to tobacco Rubisco activase and visualized using alkaline phosphatase conjugated to a secondary antibody.

When *Arabidopsis* plants were exposed to a moderate heat stress of 37.5 °C, net photosynthesis in air was inhibited to a similar extent in wild-type and rwt43 plants, but inhibition was much greater for the rwt46 plants (Table 1). These results were similar to those reported previously by Kim and Portis (2005). Net photosynthesis in the two new lines with reduced levels of activase, Δ43 and ΔDa, was also much more sensitive to inhibition by moderate heat stress than plants expressing wild-type levels of activase. The ΔDa plants were the most sensitive to moderate heat stress, exhibiting net CO_2 evolution in the light in air at 37.5 °C. For all transgenic lines, transpiration rates increased at the higher temperature, indicating that stomatal conductance was not reduced under moderate heat stress (data not shown).

The effect of moderate heat stress on photosynthesis was also examined at 9.6 mbar O_2 to minimize interference

Table 1. *Effect of temperature on the rates of net photosynthesis at air levels of CO_2 and either ambient (202 mbar) or low (9.6 mbar) O_2 in wild-type and transgenic* Arabidopsis *plants*

The numbers in parenthesis indicate the percentage inhibition at 37.5 °C.

Plant line	Net photosynthesis (μmol m^{-2} s^{-1})			
	Ambient O_2		Low O_2	
	23 °C	37.5 °C	23 °C	37.5 °C
Wild type	15.9±0.6	9.2±0.4 (42.1)	25.5±0.3	16.3±0.4 (36)
rwt43	15.9±0.3	8.6±0.4 (45.9)	ND[a]	ND
rwt46	11±0.5	1.3±0.2 (88.2)	13.9±0.5	4.0±0.6 (71.2)
Δ43	8.5±0.1	0.6±0.3 (92.9)	15.0±0.8	5.6±1.5 (62.7)
ΔDa	6.7±0.4	−1.53±0.1 (100)	13.6±0.8	2.4±0.2 (82.3)

[a] ND, not determined.

from photorespiration and to eliminate the possibility that ribulose 1,5-*bis*phosphate (RuBP) regeneration was limiting for photosynthesis under moderate heat stress (Wise *et al*., 2004). Net photosynthesis was inhibited at 37.5 °C under low O_2, although the extent of inhibition was less than at air levels of O_2. Net photosynthesis in transgenic lines that expressed reduced levels of either isoform of activase exhibited greater sensitivity to inhibition by moderate heat stress than in wild-type plants with normal amounts of activase. As in air, net photosynthesis of the ΔDa plants was the most sensitive to inhibition by moderate heat stress.

The temperature response of the operating efficiency of photosystem II (Φ_{PSII}) was determined in the wild type and the transgenic lines as an additional and independent measure of the relative sensitivity of photosynthesis to inhibition by elevated temperatures. For both wild-type and rwt43 plants, gradual temperature increases up to 45 °C had little effect on Φ_{PSII} (Fig. 2). By contrast, Φ_{PSII} decreased precipitously as the temperature increased above 35 °C in transgenic lines rwt46 and Δ43 with reduced levels of activase. At 23 °C, the Φ_{PSII} was already at the low value of 0.45 in the ΔDa plants, making it difficult to measure further decreases in Φ_{PSII} with temperature.

Response of Rubisco activation to moderate heat stress

To determine if the inhibition of photosynthesis by moderate heat stress is accompanied by changes in Rubisco activation, the initial extractable activity and fully carbamylated activities of Rubisco were measured in wild-type *Arabidopsis* and the transgenic lines. At 23 °C, initial Rubisco activity was markedly reduced in the three transgenic lines with reduced rates of net photosynthesis and lower levels of activase protein compared with the wild type, but not in the rwt43 line that had sufficient activase for wild-type rates of net photosynthesis (Table 2). The ΔDa plants had the lowest initial activity, whereas the rwt46 and Δ43 plants had similar activities equivalent to ~60% of the wild-type.

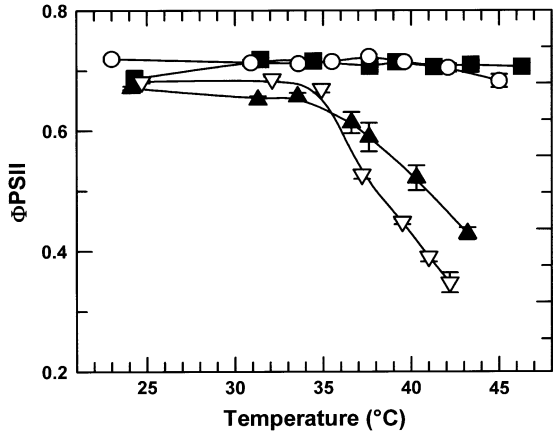

Fig. 2. Effect of temperature on the quantum yield of PSII in wild-type and transgenic *Arabidopsis* plants. The quantum yield of PSII (Φ_{PSII}), measured as $(F'_m–F_t)/F'_m$ (Maxwell and Johnson, 2000), was determined after 20 min at each temperature for wild type (filled squares), and the rwt43 (open circles), rwt46 (filled triangles), and Δ43 (open inverted triangles) *Arabidopsis* transgenics.

At 23 °C, the activation state (initial/total activities) of Rubisco was <100% in the rwt46, Δ43 and especially the ΔDa transgenic plants. By contrast, the initial activities of wild-type and rwt43 plants were slightly greater than the 'total' activities measured after incubation for 5 min or 10 min with high CO_2 and Mg^{2+} to carbamylate the enzyme 'fully'. Various control experiments including extract dilution experiments, time-course experiments, inclusion and omission of protease inhibitors, and experiments involving pre-incubation of RuBP with components of the extraction media failed to resolve the question of how Rubisco activation in wild-type and rwt43 plants could be >100% (data not shown). However, findings somewhat related to these were reported by He *et al*. (1997). These investigators presented evidence for an influence of activase in promoting the activity of carbamylated Rubisco. The data presented here also suggest the possibility that Rubisco might assume a 'hyperactive' conformation *in vivo* that is slowly lost upon extraction and dilution of Rubisco. If so, very rapid extraction and assay of initial activity might 'capture' a form of the enzyme that is subsequently lost upon incubation.

The initial activity of wild-type *Arabidopsis* and the four transgenic lines was considerably lower after a 1 h exposure to moderate heat stress compared with the control temperature. As shown previously for cotton, wheat, and oak (Feller *et al*., 1998; Haldimann and Feller, 2004), total activities were largely unaffected by exposure to 37.5 °C (Table 2). Thus, changes in initial activity mirrored changes in the activation state. At both 23 °C and 37.5 °C, the activation state of the ΔDa plants was lowest among the transgenic lines, while rwt43 exhibited the highest activation state, even higher than the wild type. Rubisco activation in rwt46 and Δ43 plants was similar at 23 °C, but was reduced to a greater extent by moderate heat stress in the rwt46 plant than in the Δ43 plants.

Table 2. *Effect of temperature on the initial activity and activation state of Rubisco in wild-type and transgenic* Arabidopsis *plants*

Plants were exposed to the indicated temperatures in air for 1 h prior to sampling.

Plant line	Initial Rubisco activity (U mg protein^{-1})[b]		Activation state[a] (%)	
	23 °C	37.5 °C	23 °C	37.5 °C
Wild type	0.46±0.03	0.26±0.01	105±1	62±2
rwt43	0.50±0.01	0.33±0.01	117±3	85±6
rwt46	0.31±0.01	0.12±0.01	87±3	32±1
Δ43	0.27±0.02	0.18±0.01	78±2	43±4
ΔDa	0.24±0.01	0.11±0.01	55±2	25±3

[a] Initial/total activity.
[b] A unit is 1 μmol CO_2 fixed min^{-1}.

Discussion

The essential role of activase in maintaining Rubisco in an active conformation was initially established in studies of an *Arabidopisis* mutant deficient in activase protein (Salvucci *et al*., 1985, 1986). The results were later verified using antisense technology to reduce the level of activase in transgenic *Arabidopsis*, tobacco, rice, and *Flaveria* plants (Mate *et al*., 1993; Jiang *et al*., 1994; Eckardt *et al*., 1997; Jin *et al*., 2004; von Caemmerer *et al*., 2005). In every case, transgenic plants with drastically reduced amounts of activase had lower rates of net photosynthesis, even in atmospheres containing elevated CO_2. Since the central function of activase is to keep Rubisco active sites free of tight-binding inhibitors, these results indicate that when the amount of activase is reduced below a critical threshold amount, the remaining activase is insufficient to service the available Rubisco molecules.

Direct measurements of xylulose *bis*phosphate synthesis have shown that the rate of catalytic misfire by Rubisco increases with increasing temperature to at least 50 °C, exhibiting the same Q_{10} as the increase in catalytic activity (Salvucci and Crafts-Brandner, 2004*b*). This epimer of RuBP, which is produced directly at the active site by misprotonation of the enediol reaction intermediate (Edmonson *et al*., 1990), inactivates Rubisco by trapping sites in a closed, dead-end complex. This complex is slow to open without the intervention of activase. It is proposed that Rubisco deactivates at elevated temperatures because the increased rates of synthesis of xylulose *bis*phosphate and possibly other (Kane *et al*., 1998; Pearce and Andrews, 2003) catalytic misfire products are not offset by faster rates of reactivation by activase. Recently, Kim and Portis (2006) showed that the rate but not the final extent of Rubisco deactivation increases with temperature, but the differences were less significant at physiological concentrations of Mg^{2+}. These investigators suggested a role for increased decarbamylation in the deactivation of Rubisco with temperature, particularly at physiological concentrations of Mg^{2+} (Kim and Portis, 2006).

Based on the relationship between activase amount and photosynthesis described above, it would be expected that photosynthesis in plants with reduced amounts of activase should exhibit greater sensitivity to inhibition by moderate heat stress than in plants with wild-type amounts of activase. With less activase to service the available Rubisco, even slight increases in misfire product production with temperature would cause Rubisco to deactivate and photosynthesis to decline more precipitously in plants with reduced amounts of activase. Exacerbating the decrease in Rubisco activation is the relatively low temperature optimum of activase. A lower activase activity at elevated temperatures would reduce the rate of reactivation, further increasing the rate of Rubisco deactivation (Salvucci and Crafts-Brandner, 2004*a*, *b*). Thus, the greater sensitivity of photosynthesis to inhibition by moderate heat stress observed with the rwt46, Δ43, and ΔDa plants is entirely consistent with a proposed mechanism for photosynthetic inhibition based on relative rates of deactivation and reactivation. That photosynthesis is more thermotolerant in transgenic tobacco plants with reduced amounts of Rubisco (SJ Crafts-Brandner and ME Salvucci, unpublished data) provides further support for this mechanism. Compared with the wild type, the higher ratio of activase: Rubisco in these plants should be capable of offsetting the faster rates of Rubisco deactivation at higher temperatures.

Stromal oxidation accompanies moderate heat stress and is thought to be a consequence of thylakoid leakiness (Schrader *et al*., 2004). Based on this observation, Schrader *et al*. (2004) suggested that a more oxidized stroma inhibits photosynthesis, possibly by inhibiting activase. However, Kim and Portis (2005) showed that photosynthesis in transgenic *Arabidopsis* plants with thylakoid membranes that are less permeable to ion leakage due to alterations in the fatty acid composition showed no improvement in thermotolerance. Measurements of the activation state of malate dehydrogenase indicated that stromal oxidation initially increased in wild-type *Arabidopsis* plants when the temperature was increased from 25 °C to 30 °C, but remained constant with additional increases in temperature (Kim and Portis, 2005). Thus, direct evidence for membrane leakiness during moderate heat stress is lacking, and the response of stromal oxidation to temperature does not always correlate with inhibition of photosynthesis.

The data presented here and in previous studies also argue against the proposed involvement of stromal oxidation as a causative agent in the temperature inhibition of activase (Schrader *et al*., 2004). While the redox-sensitive disulphide of the α-isoform of activase could be adversely affected by increased stromal oxidation, the results with transgenic *Arabidopsis* lines expressing suboptimal levels of either the 43 (Δ43) or 46 kDa (rwt46) activase isoform showed that photosynthesis in both lines was sensitive to

inhibition by moderate heat stress. Similarly, Rubisco activation decreases under moderate heat stress in plant species such as tobacco and maize that express only the non-redox-regulated isoform of activase (Crafts-Brandner and Salvucci, 2000, 2002). Thus, plants that expressed the form of activase that is insensitive to changes in redox state were affected by moderate heat stress in a similar manner to plants that expressed just the redox-sensitive form or both forms of activase. Photosynthesis and Rubisco activation in the Δ43 and rwt46 lines were much more sensitive to moderate heat stress than in the wild type, indicating that the amount rather than the isoform was the important factor for determining heat sensitivity. It should be noted that Rubisco activation was slightly more sensitive to moderate heat stress in the rwt46 compared with the Δ43 plants, and net photosynthesis was more sensitive to inhibition by moderate heat stress under low O_2 conditions. This result is consistent with the finding that the α-isoform is the more heavily regulated of the two activase isofoms (Zhang and Portis, 1999; Zhang *et al*., 2002) and could reflect a slightly greater sensitivity of this form of activase to changes in redox, ATP/ADP ratio or other conditions in the chloroplasts.

The lowest photosynthetic rates and Rubisco activation state were observed with the ΔDa plants that expressed activase from the Antarctic hairgrass. Even though the amount of activase in these plants was similar to the amount in the Δ43 plants, their growth and photosynthesis rates were significantly slower under control temperatures. In addition, photosynthesis and Rubisco activation in the ΔDa plants exhibited greater sensitivity to inhibition by moderate temperature than in either of the transgenic lines expressing reduced amounts of *Arabidopsis* activase. While it is tempting to speculate that the greater sensitivity of the ΔDa plants was caused by the more thermolabile activase in these plants (Salvucci *et al*., 2004*c*), direct measurement of the ability of purified *D. antarctica* activase to activate *Arabidopsis* Rubisco showed that the interaction with Rubisco was somewhat compromised (see supplementary material at JXB online). Reduced interaction with Rubisco would be tantamount to reducing the amount of activase, which the present results show would lead to greater heat sensitivity and would explain the poor performance of the ΔDa plants under control temperatures. Thus, future attempts to improve the thermotolerance of activase will require alterations in activase structure that improve its temperature response without adversely affecting its interaction with Rubisco.

Supplementary material

Data showing that purified activase from *D. antarctica* was less effective than purified activase from *Arabidopsis* in activating *Arabidopsis* Rubisco is available as supplementary material at JXB online. Recombinant versions of the same forms of the enzymes that were expressed in the ΔDa and Δ43 transgenic plants, i.e. the β-isoform of *D. antarctica* activase and the β-isoform of *Arabidopsis* activase containing an S-Tag, respectively, were used for the comparison.

Acknowledgement

The authors wish to acknowledge the technical assistance of Nancy Parks.

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